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MUNUTES OF THE PACIFIC NORTHWEST SYMPOSIUM (4TH) HELD IN PORTLAND, OREGON ON SEPTEMBER 4, 1968

E. F. Eldridge

Public Health Service
Portland, Oregon

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MINUTES OF THE

FOURTH SYMPOSIUM, PACIFIC NORTHWEST

Arranged by E. F. Eldridge
U. S. Public Health Service
Portland, Oregon
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A symposium on "Short-term Bioassay" was held in Room 104, U. S. Court House, Portland, Oregon, on September 4, 1958. It was attended by 44 persons representing industry, pollution control regulatory agencies, fisheries and game agencies and educational institutions. A list of those attending the meeting, their connections and addresses is attached to these minutes.

The following is the agenda used: (The persons indicated led the discussions in each case)

1. Purpose of the symposium - E. F. Eldridge
2. Toxic wastes and the aquatic environment - Glenn Carter
3. Bioassay as a measure of toxicity - Jerry Stein
4. Acute and chronic toxicity - R. L. Foster
5. Procedures and equipment for short-term bioassay - Peter Doudoroff
6. Application factors - Peter Doudoroff
7. Monitoring industrial effluents - E. F. Eldridge
8. Possible use as criteria - Vinton Bacon
9. Some experiences with the use of the method of bioassay - R. A. Wagner

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An outline of the opening remarks by the item leader and the discussions under each item follows:

1. Water pollution control is a conservation measure - conservation of the quality of this area's most vital resource, water. Adequate and reasonable pollution control requires a delicate balancing of factors, many of which are not related to water quality and which may be economic.

Because of the impact of pollution control on the health and welfare of the people and the economy of the area, a more specific knowledge and understanding of the technical aspects of the pollution problem than is now available is essential.

Present knowledge is not adequate for the proper administration of pollution control programs. The area where knowledge is most inadequate is in the methods of measurement, especially of the biological effects due to toxicity.

The development of industrial, agricultural and household chemicals has reached extensive proportions. Many of these chemicals are highly toxic and are chemically complicated. Chemical tests are either not available or may not be adequate in the concentrations involved. Chemical tests do not indicate degree of toxicity, since it varies with conditions such as temperature, alkalinity and the presence of other ions or compounds. Toxic compounds may be synergistic or antagonistic when present in the same water.

Since the effects of toxicity are largely biological, it is natural to turn to bioassay as a means of measuring these effects. Bioassay methods are not without objections.

Toxic effects may be acute or chronic and are measured by short and long-term bioassay respectively. Both types of measurement are important, but the former will be emphasized here.

A major objective of the symposium is to stimulate the use of bioassay and encourage its further development.

2. Some examples of the effects of toxic wastes on the aquatic environment were cited:

a. About 800,000 acres of forest land was treated with DDT in diesel oil for control of the spruce budworm. The rate of application was 1 bl/acre. Largely because of the method of application the DDT reached a tributary of the John Day River killing all fish and bottom organisms in a substantial area.

b. Dowcide incorporated in hardboard for termite control destroyed all life in an Oregon stream for two miles below the discharge.

c. A kill occured when Premerge, a Dow Chemical Company product was used to kill weeds in bean patches. Carelessness in preparing the spray allowed the chemical to enter the stream.

d. Detergents from a laundry caused a kill in the Necanicum River, Oregon.

e. Resin soaps were a possible factor in a fish kill on the Clark Fork River in Idaho.

The above listed kills were not confined to fish but involved a large group of organisms.

The results of fish kills are usually based on circumstantial evidence rather than experimentation. This can be corrected by adopting a definite procedure in the investigation of fish kills.

Oregon does not have a written precise procedure for investi gating fish kills such as adopted by the Ohio River Sanitation Commission. Neither does Washington. There was a feeling that there is such a variation in each kill that a precise procedure would only delay the investigation. However, there should be an arrangement by which the control agency is promptly informed of the kill. The investigation should be made by competent personnel.

3. The bioassay as a measure of toxicity was discussed. One disadvantage cited was that responses of laboratory animals to the test materials are not always clear cut. Some animals are weak, others tolerant. Oysters and other shellfish present some unusual laboratory difficulties in this connection.

The terms LD_{50} (lethal dose - 50%) and TL_m (median tolerance limit) were discussed. The reason for the adoption of the TL_m method in bioassay work originated from studies of the effect of temperature. Whereas the LD_{50} refers to a certain concentration of a substance in water that kills 50% of the test organisms, the TL_m refers to anything which may be lethal, including temperature, pH, and the like.

It is important to consider the broad physiological spectrum of an organism in response to a toxicant, rather than simply the mortality of the organism in contact with that substance.

The worth of the short-term bioassay was question in that

none of the vital activities of an organism can be determined by bioassay. It was agreed, however, that the short-term bioassay has probing value. Wherever industry has undertaken tests, they have gone into long-term tests.

4. Acute vs. Chronic Toxicity differs in the time involved in the test. Chronic toxicity effects may require years to show. Between the two there is a "gray" zone.

Administration and budget are very different in that an acute test may be made for a few dollars, whereas a long-term test usually requires thousands of dollars (as high as $20,000) owing to time involved, more expensive equipment, and the like.

As to the kinds of situations which require either acute or chronic tests, the acute tests are suggested where there are occasional discharges of a toxicant. Washing of insecticides into streams is an example. Chronic or long-term type tests may be involved in the case of heavy industries, laundries and the like where continuous discharges take place. In such instances long-term tests are suggested to determine maximum permissible concentrations of wastes which may be discharged to water courses. Chronic tests are valuable where there are situations which produce subtle damage in contrast to the spectacular type of thing known as a fish kill. In the long run subtle damage may be even more deleterious to an aquatic environment than a sudden spectacular fish kill.

Some of the areas where chronic tests have advantages over acute short-term tests are:

a. Application factors may be applied with greater accuracy.

b. Detection of cumulative poisons may be made.

c. Variability in tolerance at different stages in the life cycle of an organism can be determined.

d. Delayed effects may show up.

e. Synergistic or antagonistic effects may require an extended period of time.

The disadvantage of such a test are:

a. The high-budget costs - from $5,000 to $20,000 for a single test.

b. The time element. Minimum time for a long-term test is 6 to 8 weeks. In the case of salmon as a test organism, the sensitive state of salmon used as test animals may occur at only a short period each year and may limit the number of tests to one per year.

c. The need for expensive equipment. Continuous flow type of apparatus is usually necessary because of the accumulation of waste products; therefore, precision pumps, flow rate meters, temperature controls, etc. are needed.

d. It is necessary to feed fish or test animals, where this is not necessary in a short-term test.

e. Problems of fish diseases.

f. Changes of personnel will not be too serious with the short-term tests, but will be critical in the case of the long-term tests.

g. Growth rate problems. As the fish grow it is necessary to reduce their number in a given tank or trough in order to maintain a more or less constant total weight of organisms.

5. Discussion of short-term bioassay methods - It was agreed that short-term bioassay may be used as a tool in water pollution control and in the study of toxic wastes. It can be used more or less routinely as a control activity. It is important to create conditions in the laboratory which simulate those found in the field insofar as possible. Also, it is desirable to have reproducible results.

Standardization of methodology is necessary. Reprints of the paper "Bioassay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish" were passed out to the members in attendance. Details of the procedure are covered in the paper referred to above. The following points were discussed:

a. Use of suitable diluent water - here it is important to use the water into which the wastes are to be discharged or one having the same characteristics. Either waters above all waste discharges uncontaminated or waters from immediately above the plant itself should be used.

b. Control of temperature is not as important as in the case of the long-term tests. However, the temperature range should be somewhat restricted.

c. A satisfactory dissolved oxygen concentration should be maintained.

d. The duration of tests should be at least 48 hours, preferably 96 hours, with observations at 24, 40, and 48 hours. The use of the logarithmic series scale is best in plotting the TL_m.

6. The chief value of the short-term bioassay is its use as a probing tool to determine within a plant which particular fraction or fractions of wastes are most highly toxic. Also, the method has use in the field in the immediate area of a plant to trace the movement of plant discharges and to determine the cause of acute toxicity in fishes and aquatic life. However, there are certain limitations in the use of the data from short-term bioassay. If the data are applied directly there is no safety margin provided in that the TL_m refers to a concentration which will kill 50% of the test fish. To complicate the picture, there is a wide variation in tolerances of the different test organisms. In order take these factors into account the use of some kind of application factor is important. The Ohio River Sanitation Compact known as ORSANCO has adopted a conservative and tentative application factor of one-tenth of the 48-hour TL_m.

The importance of development of slimes, trace elements, etc. which have profound effect upon toxicity was stressed.

No conclusion was reached regarding the application factors to be applied except that they should be patterned to each situation. Generally applied application factors are not realistic.

More research and field application are necessary in order to stimulate the use of bioassay.

7. The following are some of the objectives of a monitoring program.

a. To provide industrial management with information as to whether or not the wastes from their operations exhibit toxic characteristics and, if so, the magnitude of the toxicity and the source of the toxicity.

b. To provide a basis on which to develop means of eliminating or reducing toxicity by alterations or changes in operational procedures.

c. To determine the need for treatment facilities and, if so, the type of treatment and capacity of structures required.

d. To provide the control agency with the necessary information on which to base reasonable and effective requirements.

Three out of the four objectives refer to the use of the results by industrial management. It is always desirable that management should take the initiative as regards their waste disposal problem. Too often industry knows too little about their wastes. Often they depend upon the regulatory agency to furnish the information for them. The agency should help and advise, but the industry should keep on top of their own problems. The industry's interest should stem from top levels.

In monitoring an industrial waste, it is usually advisable to conduct a joint activity, industry, and agency. In this way controversies and arguments can be largely avoided. Also, the competence of the two groups will be combined.

In all cases where there is a question of whether or not the waste from an industry is toxic, management should initiate some type of bioassay with or without the agencies assistance. Very often correction is simple. The source of the toxicity may be in a minor operation.

The bioassay should not be confined to the effluent. If the effluent is found to be toxic, assays should be made on the wastes from various operations, if the nature of the process allows such a breakdown.

8. The principal aim of bioassay is to find a safe concentration or concentrations of different substances which will be compatable with water uses. A series of questions were presented which relate to the use of bioassay as a water quality criteria:

a. "What do you have after you have conducted a test and determined short-term bioassay?" One answer was, "An educated guess." It was the concensus of the group that you would have a fair indication of the relative toxicity of the water or waste tested.

b. "If we test only one animal, can we be on safe ground or how many animals do we have to test in order to have our test reliable?" Several of those present agreed if the results referred to the type of organism required protection (fish or shellfish), the rest of the food chain will more or less take care of itself.

In contrast to this point of view it was suggested that application factors will have to be based on long-term effects through studies on artificial streams or actual streams and that productivity will have to be the criteria rather than bioassay results. If certain organisms in the food chain are eliminated by a given waste and other

organisms can take their place so that the over-all productivity is not adversely affected, then the end product to be protected should likewise not be affected.

c. "Have we the correct test animal in the fish or would the entire food chain be better served by the use of three test animals such as a shrimp, a fish and a diatom?" The discussions indicated that there was no reason for the selection of the three types of organisms. However, it was agreed that there is no certainty that the correct test animal has been found.

d. "Why do biologists who have worked in their field of bioassay join the vanguard of the 'doubting Thomases' when the chips are down?" It is discouraging to find that the very people working with these problems case the most doubt on their own work. It is necessary to take a calculated risk in the discharge of wastes. If productivity is observed to go down, it is then necessary to seek the solution by more stringent control.

If bioassays are to be used as criteria for water quality, the "multiple use concept" must be applied. The biologist in the very near future must use the results available and tie them into this "multiple use concept."

It will probably prove necessary in some cases to "dedicate" certain areas in the immediate vicinity of outfalls for dispersion and diffusion of waste. What effect will these dedicated areas have on fish swimming through the area? Will the effect be extended to other areas? These and other questions will have to be answered by biologists.

No decision was reached regarding the use of bioassay as water quality criteria. No substitute, however, was offered. Chemical tests for the most part do not serve as criteria. With the exception of pH, dissolved oxygen and some substances whose toxicity is not affected by water conditions, the only hope for adequate criteria is the bioassay supported by a continuing study of productivity. General criteria are not feasible.

8. The final discussion involved a description of experiences with the use of short-term bioassay in field work. These experiences as related by a state agency representative clearly indicated the value of the short-term bioassay in tracing the source of acutely toxic substances in the field and in finding the source of the toxic component or a waste within an industry. The discussion of these field experiences demonstrated the value of the short-term bioassay as a tool in the control of toxic industrial wastes.